REMARKS

It is respectfully requested that this application be reconsidered in view of the above amendments and the following remarks and that all of the claims remaining be allowed.

Claims Amendments:

Claims 15 and 25-27 have been canceled without prejudice or disclaimer. Applicants specifically reserve the right to file at least one continuing application directed to the canceled subject matter.

Claim 10 has been amended to depend from claim 1. Claims 16-24 have been amended to depend from claim 10 instead of claim 15. Claims 12 and 23 have been rewritten to independent forms.

No new matter has been added by these amendments. The Examiner is hereby requested to enter these amendments.

Objection

The objection of claims 12-13 and 23-24 as being of improper dependent form has been obviated by amendments of claims 12 and 23. Thus, claims 12 and 23 have been amended to be independent claims, and claims 13 and 24 now depend only from claims 12 and 23, respectively. Therefore, the objection is now moot, and its withdrawal is respectfully requested.

Rejection Under 35 U.S.C. §112, First Paragraph (Enablement):

The rejection of claims 10 and 14-27 under 35 U.S.C. §112, first paragraph, as allegedly not being enabled is obviated-in-part and traversed-in-part as set forth below.

1. Claims 10 and 16-24

Claim 10, as amended, is dependent from claim 1 and thus directed to an isolated antibody, or antigen binding fragment thereof, that specifically binds to a 499E9 polypeptide having the amino acid sequence as shown in SEQ ID NO: 2. Claim 10 further recites that the antibody is raised against a polypeptide selected from the group consisting of:

a) a substantially pure or recombinant 499E9 polypeptide exhibiting 100% sequence identity over a length of at least 12 contiguous amino acids to SEQ ID NO: 2;

b) a natural sequence 499E9 of SEQ ID NO:2; and

c) a fusion protein comprising 499E9 sequence.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. MPEP §2164.01; United States v. Telectronics, Inc., 8 USPQ2d 1217, 1223 (Fed. Cir. 1988). Claim 10 is fully enabled under this test.

> Harlow and Lane (Antibodies: A Laboratory Manual, 1988) states:
> Peptide-carrier conjugates seldom fail to elicit a response because of tolerance. Consequently, the peptides can usually be seen as epitopes, and high-titered antisera commonly prepared...
> The two most important advantages of anti-peptide antibodies are that they *can be prepared immediately after determining the amino acid sequence* of a protein (either from protein sequencing or form DNA)and that *particular regions of a protein can be targeted specifically for antibody production.*" (At page 73; emphases added; copy attached.)

Moreover, knowledge of how to choose appropriate peptide sequences for antibody production is also well known in the art (see, e.g., Harlow and Lane, *supra*, at pages 75-76; copy attached). Thus, the subject specification and knowledge well known in the art enable production of antibodies directed to 499E9 peptides comprising at least 12

contiguous amino acids of SEQ ID NO: 2. Therefore, the subject invention as recited in claim 10 is clearly enabled.

As stated in the Office Action, the specification is enabling for antibodies or fragments thereof which specifically bind SEQ ID NO:2 (page 3, paragraph number 9 of the Office Action). Claim 10 encompasses a subset of these antibodies or fragments that have been deemed enabled, and methods of preparing and using this subset are sufficiently disclosed in the present application. Specifically, methods of preparing antibodies are disclosed, for example, at pages 23-26 and in Example 5. The specification further provides natural sequence 499E9 proteins (such as SEQ ID NO:2), functional variants and physical variants of 499E9 as well as methods of preparation or purification thereof (see, for example, pages 15-23), and methods for amino acid sequence comparison (e.g., at page 16, first full paragraph). Therefore, a skilled artisan can readily prepare or isolate polypeptides that exhibit 100% sequence identity over a length of at least 12 contiguous amino acids with SEQ ID NO: 2, natural sequence 499E9 proteins, or fusion proteins comprising the 499E9 sequence, and use these polypeptides to raise antibodies. It is also within the knowledge in the art to examine if the resulting antibodies specifically bind SEQ ID NO:2, for example, by double diffusion analysis. Therefore, a person of reasonable skill in the art can make or use the claimed invention without undue experimentation.

Similarly, claims 16-24 are also enabled. Claims 16-22 have been amended to depend from claim 10, and claims 23-24 have been rewritten to incorporate all the elements of claim 10. As such, claims 16-24 correspond to claims 2-9 and 11-13, which have been deemed allowable. Therefore, claims 16-24 are enabled as well.

Accordingly, Applicants respectfully request withdrawal of this part of the rejection.

2. Claims 14, 15 and 25-27

Claim 14 has been amended to recite "the polypeptide of SEQ ID NO:2" instead of "a polypeptide of SEQ ID NO:2". Accordingly, claim 14 does not read on subsequences of SEQ ID NO:2 and is fully enabled. Claims 15 and 25-27 have been canceled without prejudice or disclaimer. Therefore, Applicants respectfully request that the rejection be withdrawn with respect to these claims.

Rejection Under 35 U.S.C. §112, First Paragraph (Written Description):

The rejection of claims 10 and 14-27 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such as way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention, is obviated-in-part and traversed-in-part for the following reasons.

As described above, claim 10 is dependent from claim 1 and thus directed to an isolated antibody, or antigen binding fragment thereof, that specifically binds to a 499E9 polypeptide having the amino acid sequence as shown in SEQ ID NO: 2. Claim 10 further recites that the antibody is raised against a polypeptide selected from the group consisting of:

a) a substantially pure or recombinant 499E9 polypeptide exhibiting 100% sequence identity over a length of at least 12 contiguous amino acids to SEQ ID NO: 2;

b) a natural sequence 499E9 of SEQ ID NO:2; and

c) a fusion protein comprising 499E9 sequence.

Pursuant to the Guidelines for Examination of Patent Applications under the 35 U.S.C. 112, first paragraph, "Written Description" Requirement (Federal Register 66 (4): 1099-1111; the "Guidelines"), possession may be shown in a variety of ways, including description of an actual reduction to practice and description of distinguishing identifying

characteristics sufficient to show that the applicant was in possession of the claimed invention. Federal Register 66 (4), at 1104.

The present specification provides sufficient distinguishing identifying characteristics to show that Applicants were in possession of the claimed antibodies and fragments thereof. A natural sequence 499E9 polypeptide (SEQ ID NO:2) is disclosed, and a skilled artisan can readily determine if a polypeptide in question exhibits 100% sequence identity over a length of at least 12 contiguous amino acids to SEQ ID NO: 2 by performing sequence comparison using SEQ ID NO:2. Similarly, the specification also describes in detail natural sequence 499E9 polypeptides having SEQ ID NO:2, or fusion proteins comprising 499E9 sequence. Moreover, claim 10 further requires that the antibody or fragment specifically recognize SEQ ID NO:2, which has been deemed to satisfy the written description requirement as evidenced by the allowability of claim 1.

Accordingly, the present application provides adequate distinguishing identifying characteristics for claim 10. Claims 16-24, which depend from claim 10 or otherwise contain all the elements of claim 10, are also adequately described. Claim 14 has been amended to recite "the polypeptide of SEQ ID NO:2" and thus does not read on subsequences. Claims 15 and 25-27 have been canceled, and thus the issue of written description is moot.

In view of the above, withdrawal of this rejection is respectfully requested.

Allowable Subject Matter

The Office Action indicates that claims 1-3, 6-9 and 11 are allowable, and claims 12-13 would be allowable if rewritten in independent form. Claim 12 has been amended to be an independent claim, and claim 13 is now only dependent from claim 12. Therefore, Applicants submit that claims 1-3, 6-9 and 11-13 are in condition for allowance, as are all other claims currently pending in this application.

Conclusions:

For the reasons set forth above, Applicants submit that the claims of this application are patentable. Reconsideration and withdrawal of the Examiner's rejections are hereby requested. Allowance of the claims remaining in this application is earnestly solicited.

In the event that a telephone conversation could expedite the prosecution of this application, the Examiner is requested to call the undersigned at (650) 622-2330.

Respectfully submitted,

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Attachment to Amendment dated September 12, 2002

Marked-up Copy

Claims

10. (Twice amended) [An isolated] The antibody of claim 1, or antigen binding fragment thereof, that [specifically binds to] is raised against a polypeptide selected from the group consisting of:

a) a substantially pure or recombinant 499E9 polypeptide exhibiting 100% sequence identity over a length of at least 12 contiguous amino acids to SEQ ID NO: 2;

b) a natural sequence 499E9 of SEQ ID NO:2; and

c) a fusion protein comprising 499E9 sequence.

12. (Amended) [The antibody, or antigen binding fragment of claim 1, that] An isolated antibody, or antigen binding fragment thereof, that specifically binds to a polypeptide having the amino acid sequence as shown in SEQ ID NO: 2, wherein the antibody or antigen binding fragment is conjugated to a detectable label.

14. (Amended) An isolated antibody, or antigen binding fragment thereof, that binds to [a] the polypeptide of SEQ ID NO:2.

16. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody is polyclonal.

17. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody is monoclonal.

18. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody is a 499E9 antagonist.

19. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antigen binding fragment is a $F(ab')_2$, Fab, or F_v fragment.

20. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody or fragment binds to the polypeptide with a K_d of at least about 30 μM.

21. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody or fragment binds to the polypeptide with a K_d of at least about 10 μM.

22. (Amended) The antibody, or antigen binding fragment of claim [15] 10, wherein the antibody or fragment binds to the polypeptide with a K_d of at least about 3 μM.

23. (Amended) An isolated antibody, or antigen binding fragment thereof, that specifically binds to a 499E9 polypeptide having the amino acid sequence as shown in SEQ ID NO: 2, wherein the antibody is raised against a polypeptide selected from the group consisting of:

a) a substantially pure or recombinant 499E9 polypeptide exhibiting 100% sequence identity over a length of at least 12 contiguous amino acids to SEQ ID NO: 2;

b) a natural sequence 499E9 of SEQ ID NO:2; and

c) a fusion protein comprising 499E9 sequence;

wherein the [The] antibody[,] or antigen binding fragment [of claim 15 that] is conjugated to a detectable label.

Antibodies

A LABORATORY MANUAL

Ed Harlow
Cold Spring Harbor Laboratory

David Lane
Imperial Cancer Research Fund Laboratories

Cold Spring Harbor Laboratory
1988

and their corresponding protein sequences have become known, synthetic peptides have been used to prepare antibodies specific for previously uncharacterized proteins (Sutcliffe et al. 1980; Walter et al. 1980; and reviewed in Lerner 1982, 1984; Walter 1986; Doolittle 1976; and in Ciba Foundation 1986). Peptides are normally synthesized using the solid-phase techniques pioneered by Merrifield (1963). The synthetic peptides are purified and coupled to carrier proteins, and these conjugates are then used to immunize animals. In these cases, the peptides serve as haptens with the carrier proteins, providing good sites for class II–T-cell receptor binding. Peptide–carrier conjugates seldom fail to elicit a response because of tolerance. Consequently, the peptides can usually be seen as epitopes, and high-titered antisera commonly are prepared. Characteristically, these antibodies will bind well to denatured proteins, but may or may not recognize the native protein.

The two most important advantages of anti-peptide antibodies are that they can be prepared immediately after determining the amino acid sequence of a protein (either from protein sequencing or from DNA sequencing) and that particular regions of a protein can be targeted specifically for antibody production. Rapid conversion from DNA sequence information to antibodies has enormous potential for application in molecular biology. Similarly, the production of site-specific antibodies has immediate implications for functional and clinical studies.

literature range from 0/4 to 3/4 of anti-peptide antibodies will bind to the native antigen. Synthetic peptide antigens are also more expensive to produce than bacterial fusion protein antigens.

Bacterially expressed antigens present a different set of problems. Some will be difficult to express in *E. coli*, presumably because of their toxic side effects. In these cases, inducible systems such as the T7 systems of Studier (see Rosenberg 1987; Studier and Moffatt 1986) are recommended. Even when high levels of the antigen of interest can be produced, there may be some instances where the protein will not be immunogenic or where the antibodies will not recognize the native protein. However, because of the larger size of the bacterially expressed protein, there is a better chance that the antibodies will bind to the native protein.

A reasonable compromise for antibody production would be: (1) If the budget is limited and/or antibodies for the native protein are essential, use fusion proteins or full-length expression in *E. coli*. (2) If the budget is large enough, try both bacterially produced immunogens and peptides. (3) If the protein is highly conserved, use peptides. (4) If site-directed antibodies are needed, use peptides or prepare large banks of monoclonal antibodies against the bacterially produced immunogens.

Designing the Peptide

Probably the most frequently asked question concerning synthetic peptides is what sequence should be used for the immunogen (reviewed in Doolittle 1986). Although there is no one correct answer, enough anti-peptide antibodies have been raised to make suggestions for peptide choices. However, preparing anti-peptide antibodies is still an empirical exercise. What works well for one immunogen may fail completely for another.

Choosing the Appropriate Peptide Sequence

With careful synthesis, coupling, and immunizations, most sequences can be used to induce antibodies specific for the peptide itself. When considering which sequence to use, most people actually want to know how likely will it be that the anti-peptide antibodies will recognize the native protein. Early work suggested that peptides containing hydrophilic amino acids (Hopp and Woods 1981, 1983; Kyte and Doolittle 1982) and proline residues were more likely to be exposed on the surface of the native protein than other sequences, and many peptides have been prepared using these criteria. In assessing the value of these criteria, hydrophilicity is required but is not sufficient to predict the surface location of a particular sequence. Many strongly hydrophilic amino acid sequences are buried in water pockets or form inter- or intramolecular bonds and are thus excluded from interactions with anti-native antibodies. Therefore, hydrophilicity can be thought of as required but not sufficient for choosing peptide sequences (see p. 661 for hydrophilicity values). Hydrophilic peptides are also more likely to be soluble for coupling reactions.

The presence of proline residues in synthetic peptides originally was suggested because β-turns often form portions of known epitopes. However, the presence of proline residues in peptides does not have much predictive value when antisera are tested for binding to the surface of native proteins. Although many excellent anti-peptide antisera have been prepared against sequences with proline residues, there is not sufficient evidence to target prolines when designing peptides.

More recently, several workers have noted that carboxy-terminal sequences often are exposed and can be targeted for anti-peptide sequences. Although using carboxy-terminal sequences does not guarantee that the resulting antibodies will recognize the native protein, a surprisingly high percentage will. Similarly, many amino-terminal regions are exposed, and these also may make good targets.

Another potentially useful parameter for selecting peptide sequences is the "mobility" of the amino acid residues. Originally, it was noted that the regions of a protein that become epitopes often have a higher temperature than other regions, as determined by NMR and X-ray structure (Moore and Williams 1980; Robinson et al. 1983; Tainer et al. 1984; Westhof et al. 1984). Higher temperature in crystallography and NMR distinguishes regions that are more mobile from

regions that are more static. These observations have led to the suggestion that stretches of amino acids that are more flexible are more likely to be epitopes. In the preparation of anti-peptide antibodies, when a peptide is coupled to a carrier molecule, it has a different local environment than in the original protein. When choosing a sequence for antibody production, a region of the protein that is more flexible will be more likely over time to form a structure that is similar to the peptide–carrier conjugate. Although the measure of mobility may become a useful criterion for selecting good peptide sequences, it has not been tested in enough detail to determine whether it will have any predictive value.

At present, a reasonable order of suggestions for choosing peptide sequences would be:

1. If possible, use more than one peptide.
2. Use the carboxyl-terminal sequence if it is hydrophilic and if a suitable coupling group is available or can be added.
3. Use the amino-terminal sequence if it is hydrophilic and if a suitable coupling group is available or can be added.
4. Use internal hydrophilic regions; perhaps using longer peptides.

Size of the Peptide

The smallest synthetic peptides that will consistently elicit antibodies that bind to the original protein are 6 residues in length. Responses to smaller peptides are typically weak or will not recognize the protein of interest, either in a native or denatured state. Since epitopes consisting of smaller regions have been reported, the lower limit presumably reflects the difficulty of recognizing the smaller peptides coupled to carriers. With peptides of 6 amino acids or slightly larger, the responses vary. Some will generate good antibodies and some will not. Generally, peptides of approximately 10 residues should be used as a lower limit for coupling.

In the literature two strategies are suggested for peptide length. One school suggests using long peptides (up to 40 amino acids long) to increase the number of possible epitopes, while other authors argue that smaller peptides are adequate and their use ensures that the site-specific character of anti-peptide antibodies is retained. Both strategies have been used successfully. Two important preliminary questions to consider are: (1) Does the anti-peptide serum need to recognize the native protein? If so, use longer peptides or prepare anti-peptide antisera against multiple peptides. (2) How good is your peptide synthesis facility? Peptides over 20 residues in length are increasingly difficult to synthesize, yielding products with inappropriate side reactions. Longer peptides also are more likely to contain residues that make the coupling to carrier molecules more difficult. The correct decison between peptides with 10–15 residues and longer peptides will depend on the experimental design and will normally be a compromise between these factors. The safest choice, but also the most expensive, will be to prepare multiple small peptides of 10–15 amino acids in length from various regions of the sequence.